Morphology and Histochemistry of the Glandular Trichomes of Lippia scaberrima (Verbenaceae)

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INTRODUCTION
The genus Lippia (Verbenaceae) is extremely widespread, encompassing about 240 species, many of which are aromatic, and occur mainly in the tropics of America and Africa (Pascual et al., 2001). A large number of species are commonly used for their medicinal properties and as seasonings in food preparation. The intriguing discovery of hernandulcin, a sesquiterpene rated about 1000 times sweeter than sucrose, in plant extracts of the sweet herb Lippia dulcis, has highlighted the possibility of using this compound as a prototype for a new generation of food sweeteners (Catalan and de Lampasona, 2002). Traditional medicinal uses of Lippia species include the treatment of a variety of ailments. However, according to Morton (cited by Pascual et al., 2001) the majority of species are used as remedies for gastrointestinal and respiratory complaints. In South Africa, the five indigenous Lippia species have ethnopharmacological application, and are well known to Tswana, Pedi, Zulu and Xhosa communities (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997). One of these species, Lippia scaberrima, is an aromatic, perennial shrub, which reaches a height of about 60 cm and prefers to grow in a dry climate with summer rainfall (Retief and Herman, 1997). Medicinal teas made from leaves of L. scaberrima are sold commercially as a general health tea under the name ‘Mosukujane tea’.

The activity of medicinal plants can be attributed to their secondary metabolites, some of which occur in the essential oil of aromatic plants. In L. scaberrima the oils were found to contain limonene and carvone as main components (Combrinck et al., 2006). However, intra-species variation in the chemical composition of the oil is common for the genus Lippia (Catalan and de Lampasona, 2002). Glandular structures are known to be primary sites of secondary metabolite biosynthesis, secretion and storage and generally consist of either simple subcutaneous glands or of trichomes (Weiss, 1997). Essential oils are produced within such structures and may consist of mono-, sesqui-, di- and triterpenes, as well as flavone and isoflavone aglycones (Bisio et al., 1999). Subcutaneous glands, for example, are found in Eucalyptus, Melaleuca as well as Citrus spp. and can be observed as cavities or ducts in the epidermis. According to Murtagh and Curtis (1991), this type of gland is less likely to lose oil through evaporation than more complex trichome-type glands such as those exhibited by Mentha and Pelargonium species (Oosthuizen and Coetzee, 1983; Maffeii and Codignola, 1990). This evaporative probability may be linked to the superficial locality and therefore propensity to desiccation of trichome-type glands.

The distribution and structure of trichomes on plant surfaces contribute to the control of transpiration and temperature of the organ on which they occur (Bosabalidis, 2002). In addition, it has been postulated that the trichome density and phenolic compounds formed in these structures afford the organ protection against UV-B radiation, which results from exposure to sunlight (Liakoura et al., 1997). Superficial structures have been shown to act as pest
deterrents which obstruct ovipositioning and feeding habits of insects on leaf surfaces (Handley et al., 2005). Further insect behaviour modification by trichomes is achieved by glandular secretions, which either function in plant defence or act as attractants to insects to facilitate pollination (Weiss, 1997; Bosabalidis, 2002).

Two morphological types of glandular trichomes can be distinguished, i.e. 'capitate' or 'peltate' trichomes (Maffei and Codignola, 1990). The same basic morphology applies to both types, where they consist of a basal region, a stalk and a head. The morphological distinguishing character of a capitate trichome is the head-like shape of the gland, like the head of a pin or a floral stigma as described by Beentjie and Ghazanfar (2003). These authors identified peltate glands by their round head cell that, depending on the species, may be centrically or acen-trically attached to the stalk. This basic definition has been expanded to include variable and extraordinary trichome structures such as the flattened, disc-shaped wings of Tillandsia species (Benz and Martin, 2005). Capitate trichomes are frequently smaller than their counterparts, often occurring in denser populations and are structurally variable (Bosabalidis, 2002). In peltate glands it has been found that the stalk and base generally consist of only one cell, although the number of cells comprising the head may differ between species.

Trichome secretions may have economic value, e.g. the bulk of the essential oil of Mentha piperita occurs in peltate trichomes (Sharma et al., 2003). The importance of structural aspects is made evident by M. piperita where those specimens that exhibit more head cells, have been shown to produce more oil. Maffei et al. (1989) extracted oil from individual peltate glands using microcapillary tubes and analysed the extracts with GC-MS. They reported striking differences in the monoterpene composition among individual trichomes as well as between those trichomes on the adaxial and those on the abaxial side of the leaf.

There has been renewed interest in the use of histochemical techniques to study the nature of trichome contents. Ascensão et al. (1999) investigated the main secretion compounds of Plectranthus ornatus using histochemical methods and found that its peltate trichomes produce terpenoids and flavonoid aglycones. Likewise, Bisio et al. (1999) used histochemical procedures to localize secreted substances within and on the surfaces of hairs of Salvia blepharophylla.

The morphology, distribution and histochemistry of the secretory structures of related families, such as Lamiaceae, and related genera, such as Lantana and Origanum, are well documented (Bosabalidis, 2002). In contrast, little information is available on the structural and ultrastructural aspects of trichomes of Lippia spp., apart from the general observation that the essential oil is produced in glandular trichomes. The economic potential of L. scaberrima in the South African context requires an in depth study of the plant morphology and chemical constituents. This investigation was therefore aimed at elucidating glandular structures of L. scaberrima, thereby contributing to available information on the genus Lippia.

To this end, imaging techniques such as scanning electron microscopy (SEM), conventional light and fluorescence microscopy, as well as staining techniques, were used.

MATERIALS AND METHODS

Plant material

Fresh aerial plant parts of Lippia scaberrima Sond. were harvested in March 2006 from the Losberg area of Potchefstroom (North West Province, Republic of South Africa, 26-41366’S, 27-55043’E; 1611 m a.s.l.). For the purpose of these investigations, mature leaves were regarded as vegetative leaves harvested from the third and fourth nodes of the plant, while recently unfurled leaves from the first node were referred to as young leaves. Microscopy investigations were performed on fresh, as well as air-dried material. Twigs were placed in an open container in a dust-free environment for 3 weeks to dry naturally. Ambient temperatures ranged from 24°C to 27°C and the relative humidity from 30 % to 50 %.

Scanning electron microscopy

A 1 : 1 mixture of 2.5 % glutaraldehyde and 2.5 % formaldehyde in 0-15 M phosphate buffer (pH 7-4) was used for fixation of sections of fresh leaves and flower heads. Sectional material was washed in the phosphate buffer and post-fixed in 0-5 % OsO4. Dehydration was done in an ethanol dilution series (30 %, 50 %, 70 %, 90 %, followed by 3 × 100 %). After critical drying (Biorad E3000, Polaron, West Sussex, UK), the samples were mounted on double-sided carbon tape on stubs. They were then plasma-coated with 10 μm gold and viewed with a JEOL840 SEM (JEOL, Tokyo, Japan). Studying the surface morphology of at least 30 leaves and 30 flowers, the distribution and density of distinctive structures were observed to be similar throughout. A more quantitative estimation of this distribution was obtained by averaging counts of these structures on four 1-mm² areas on the micrograph obtained for each plant part.

Light microscopy

Trichome morphology. Upon collection, leaves were immediately fixed in a 1 : 1 mixture of 2.5 % formaldehyde and 2.5 % glutaraldehyde in 0-15 M phosphate buffer (pH 7-4) to which 0-1 % caffeic acid was added. Small leaves (≤ 15 mm²) were fixed without further sectioning, while cross-sections (5–7 mm wide) were cut across the centres of larger leaf laminas. Both small and large leaf material was later divided into smaller pieces (5 mm²) in the laboratory and fixed for 12 h before dehydration in an ethanol dilution series (30 %, 50 %, 70 %, 90 %, followed by 3 × 100 %). This was followed by progressive embedding in L.R. White resin and final polymerization at 60 °C for 36 h. Thin sections (500 nm) of the samples were cut with Ultracut E Reichert microtome (Vienna, Austria) and mounted on glass slides by gently heating at 60 °C on a slide warmer. Oil gland structures were investigated by
staining with Toluidine Blue O at pH 5.6, a general stain for tissue structure (O’Brien and McCully, 1981).

**Histochemical investigations.** Natural Product reagent was prepared by mixing aqueous AlCl₃ solution (5%) and 0.05% diphenylboric acid-β-ethylaminoester in 10% methanol as described by Heinrich et al. (2002) for the detection of flavonoids. Entire leaves were soaked in the solution for 10 min, after which the plant material was dried on adsorbent paper, cut in half across the mid-vein and mounted in oil, both adaxial and abaxial sides facing upwards. Vanillin–HCl was prepared by dissolving 0.1 g vanillin in 100 mL concentrated HCl (Nikolakaki and Christodoulakis, 2004). This reagent is a universal indicator of various compounds, enabling differentiation between different classes of secondary metabolites such as flavonoids, terpenoids and phenyl carboxylic acids based on the colouration obtained (Wagner and Bladt, 1996). Leaves were soaked for 2 min and then dried on absorbent paper before being mounted whole. For examination of autofluorescence and staining, leaves were mounted on a glass slide, without a cover glass, and viewed directly under UV light (excitation λ365 nm; emission λ397 nm). A blue filter (BF) was used to indicate further fluorescence (excitation λ386 nm; emission λ490 nm).

**RESULTS**

**Appearance and distribution of trichomes**

Externally, a random distribution of trichomes was revealed by SEM (Fig. 1A and B) and was grouped according to their morphology. Large (approx. 50 μm in diameter) and small (approx. 20 μm in diameter) bulbous glandular structures and apparently non-glandular setae, all randomly distributed between the stomata were observed (Fig. 1B). These structures occurred on both adaxial and abaxial leaf surfaces as well as on the calyces and petals. Mature vegetative leaf surfaces displayed an almost 1:1 distribution ratio between large (24) and small (26) bulbous glands per square millimetre on both adaxial and abaxial sides. Although a higher density of glandular structures was observed on leaf veins, these were not counted. In the case of adaxial leaf surfaces, the ratio of setae to each of the bulbous glands was close to 1:12 with only two setae present per square millimetre, whilst on abaxial surfaces the ratio increased to 1:6. This is in sharp contrast to the distribution on calyces, where an average of 77 setae, 38 large and 36 small bulbous glands per square millimetre was counted. The dense and overlapping population of trichomes occurring on flower petals made it impossible to determine these ratios. Trichomes were not only discerned by their morphological shapes, but also by their surface textures (Fig. 1B). A smooth indumenent layered the large bulbous structures, while a degree of ornamentation could be observed on the small trichomes. Surface sculpturing of setae, on the other hand, consisted of ridges converging towards the apex. Small, stalked peltate glands could be observed on leaf surfaces (Fig. 1C). Post-harvest drying allowed further distinction of differences in small glandular secretory trichomes (S-GSTs). These findings were investigated in more detail using fluorescence microscopy (Fig. 1D).

![Fig. 1.](image-url)
Light microscopy further elucidated the morphology of bulbous trichomes. The leaf cross-section shown in Fig. 2 indicates the presence of several different morphological types of superficial secretory glands. This figure also denotes a multicellular capitate gland (20 μm) that is separated from the epidermis by a thick cutin layer, and consists of a single stalk cell and two to four head cells (Fig. 2A). The peltate gland (50 μm) in Fig. 2B consists of a single head cell with no stalk cell and is separated from the basal cell by a valve which is visible as a thin cutaneous area. Evacuation of these glands is evident in Fig. 2D. A double membrane consisting of the cutin and plasma membrane can be seen as blue and purple lines, respectively. Other than in superficial storage structures, accumulated metabolites were also observed in central palisade tissue, which stained purple with Toluidine Blue (Fig. 2C). An oblique section of a typical seta and the surrounding basal cells is shown in Fig. 2E. The basal cells are large with a thick cutin layer that thins as it ascends the setal walls. Although not visible on this particular section, basal cells that comprise the rostellum are clearly distinguishable from the adjacent epidermal cells. The remains of cellular contents can be seen as a blue mass accumulated in the base of the seta. Not included on the cross-section is a second small type of capitate gland (Fig. 2F). In contrast to the glandular type in Fig. 1A, this gland appears to have a valve separating the basal cell and stem cell, and a unicellular head.

Glandular contents

Within each of the three main groups of trichomes (small and large bulbous glands and setae), chemical variation in content was apparent in both young and mature leaves and is summarized in Table 1. Setal content of young leaves appears to consist of immiscible fluids with only one of these fluids exhibiting autofluorescence (Fig. 3A). In mature leaf setae, however, the content appeared localized in distinct zones, with the most intense fluorescence visible in the basal region (Fig. 3B). Seepage of metabolites to the basal area in dried material could be observed as intense fluorescence after treatment with vanillin–HCl (Fig. 3C). Crystalline exudates were found on the tips of apparently damaged setae on the surface of air-dried specimens (Fig. 3D).

Large glandular trichomes (L-GSTs) did not autofluoresce, although limited autofluorescence was visible using BF. Application of Natural Product reagent did not reveal any differences in glandular content; however, vanillin–HCl enhanced differences between colour intensities of L-GSTs.
(Fig. 3E, F). Various shades of brown, ranging from straw coloured to almost black, were discerned in L-GSTs clustered within close proximity to each other, on the same leaf surface. After 10 d of drying, many L-GSTs appeared slightly deflated, while others were visibly damaged.

On mature and young leaf surfaces, numerous S-GSTs varied in shades of blue, orange and yellow (Table 1 and Fig. 4A, B), but a higher density of blue autofluorescent glands was observed on younger leaf surfaces. It was found that both uni- and multicellular glands were present and the multicellular glands in particular, showed inconsistent fluorescence (Fig. 4B–F). Unicellular orange and blue glands exhibited intense fluorescence (Fig. 4F–J). Based on the morphological changes in trichomes observed on dried material, and using SEM, two types of S-GSTs were distinguished. Some S-GSTs underwent rapid evacuation due to desiccation (Fig. 4E), whilst a second type of S-GSTs remained distended even after the evacuation of L-GSTs was evident (Fig. 4G, H) and were still visible 3 weeks after harvest. Those S-GSTs where noticeable morphological changes were evident after desiccation did not appear to change in chemical content as the fluorescence remained consistent when using the blue filter. While shrivelled S-GSTs were found to be multicellular (Fig. 4E),

**Table 1. Summary of observations of trichomes on mature and young vegetative leaf surfaces using fluorescence microscopy before and after treatment with staining reagents**

<table>
<thead>
<tr>
<th>Leaf surface</th>
<th>Autofluorescence</th>
<th>Natural Product reagent</th>
<th>Vanillin–HCl</th>
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<tbody>
<tr>
<td>Abaxial surface</td>
<td>L-GSTs quench fluorescence with UV and do not fluoresce with BF.</td>
<td>L-GSTs vary noticeably in fluorescent intensities with BF, indicating content differences. These differences also discernable as colour variation using UV light (Fig. 3E).</td>
<td></td>
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<tr>
<td>(mature leaves)</td>
<td></td>
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<tr>
<td>Abaxial surface</td>
<td>L-GSTs quench fluorescence with UV and do not fluoresce with BF.</td>
<td></td>
<td></td>
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<tr>
<td>(young leaves)</td>
<td></td>
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<tr>
<td>Adaxial surface</td>
<td>Same observations are as for abaxial surfaces with additional multicellular orange S-GSTs present.</td>
<td></td>
<td></td>
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<tr>
<td>(mature leaves)</td>
<td></td>
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<tr>
<td>Abaxial surface</td>
<td>L-GSTs exhibit no autofluorescence under BF.</td>
<td></td>
<td></td>
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<tr>
<td>(young leaves)</td>
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<tr>
<td>Setae exhibit variable compartmentalization. Yellow autofluorescence observed in base and as a central band (Fig. 3B). This fluorescence corresponds to that observed with BF.</td>
<td></td>
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<tr>
<td>Setae have yellow fluorescent contents in base compartment. Sometimes observed in central zone.</td>
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<tr>
<td>Setae observed with an absence of fluorescence, indicating different contents to that of mature leaf setae.</td>
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<td>Basal ends of setae exhibit lack of fluorescent compounds.</td>
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<tr>
<td>Setae observed with an absence of fluorescence, indicating different contents to that of mature leaf setae.</td>
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<tr>
<td>Same observations for abaxial surfaces.</td>
<td>Orange unicusular S-GSTs occur singly in ‘glandular forests’ and fluoresce intensely with BF than in the case of Natural Product reagent (Fig. 4I, J).</td>
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<td></td>
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</tbody>
</table>

L-GST, Large glandular secretory trichomes; S-GST, small glandular secretory trichomes; BF, observations using UV light with blue filter.
distended S-GSTs appeared to consist of a single head cell only (Fig. 4G–J).

**DISCUSSION**

The essential oil from *L. scaberrima* has potential economic value (Combrinck et al., 2006) and wild plants can be utilized without danger of overexploitation, since the shrubs become invasive in overgrazed veldt (Wells et al., 1986). Detailed descriptions of trichomes are available in the literature for many commercially important genera, e.g. *Mentha* (Sharma et al., 2003), *Ocimum* (Ioannidis et al., 2002), *Origanum* (Bosabalidis, 2002) and *Pelargonium* (Oosthuizen and Coetzee, 1983). Apart from the general observation that the oil is produced in glandular trichomes, there is a lack of information on the structural aspects of trichomes of *Lippia* species. Useful chemico-morphological information regarding the content, structure and distribution of the various glandular types was derived using staining and microscopy techniques. The substantially higher density of the various trichome types observed on calyces and petals, as opposed to that on leaf surfaces, coincides with results from a study by Terblanché et al. (1998). They obtained higher oil yields from fresh flower heads than from fresh leaves, and oil yield is directly related to the distribution and density of terpenoid-bearing glands. In the case of *L. scaberrima*, whole plants must be utilized for industrial production of oil since an abundance of flowers are present throughout the growing season, and are difficult to separate from leaves. However, only leaf surfaces were examined for the purpose of this study.

A combination of microscopy techniques provided valuable leads to the characteristics of main chemical classes of possible medicinal compounds produced by *L. scaberrima*. Clear distinctions between uni- and multicellular trichomes could be made using light microscopy, which therefore served to differentiate internal structural variation. On the
other hand, SEM was useful in determining the distribution, size and shapes of external structures. The major contribution of fluorescence microscopy was the elucidation of chemical differences in glandular contents.

Previous analysis of the essential oil obtained from this plant indicated an abundance of terpenoids (Combrinck et al., 2006). Terpenoid secretion was found to be restricted to L-GSTs, while the presence of phenolic compounds in S-GSTs was indicated by various colour reactions. Natural Product reagent was used to enhance the natural autofluorescence of phenolic compounds (Andary et al., 1984). Autofluorescence is diagnostic of flavonoids, which, depending on the structural type, show dark yellow, green or blue fluorescence under UV-365 nm light (Wagner and Bladt, 1996). In addition, blue fluorescence may reveal the presence of phenol carboxylic acids and coumarins. Staining with vanillin–HCl (Nikolakaki and Christodoulakis, 2004) was introduced to avoid the effect of terpenoids masking the fluorescent behaviour of any phenolic compounds present.

Development of compartmentalization in mature setae became apparent after the segregation of what initially

Fig. 4. Micrographs of individual S-GSTs, showing the structural and chemical diversity of the trichomes and their contents. (A) Abaxial surface of a young leaf following staining with Natural Product reagent. (B) Arrows indicate S-GST’s that exhibit inconsistent fluorescence when exposed to UV light, while (C) denotes fluorescence of the same structures using a blue filter. (D) Vanillin–HCl intensified the variable colour reactions of phenolic compounds. (E) Dried, multicellular glands fluoresce intensely after staining with Natural product reagent. (F) A higher density of blue multicellular glands observed on young abaxial leaf surfaces. (G and H) Paired micrographs of small, unicellular fluorescent glands that remained fully distended 3 weeks after harvest. (I and J) Paired micrographs of unicellular glands occurring in a ‘glandular forest’. Scale bar = 50 μm.
appeared to be immiscible fluids in young setae. This observation suggested cellular differentiation at a later stage during leaf maturation (Fig. 3A, B). However, according to Cutter (1980), cell differentiation terminates during early leaf development and these observations therefore point to density differences of secondary metabolite content rather than to further structural glandular development. Observations of the basal contents exhibiting autofluorescence and enhanced fluorescence with Natural Product reagent indicated that setae are also secondary metabolite storage structures and not non-glandular in nature as initially thought. Identification of the content of these hair-like structures will further elucidate their role on organ surfaces of *Lippia* spp. Further evidence of accumulated secondary metabolites in setae was provided by the observation of an intense fluorescent reaction of dried material with Natural Product reagent, due to seepage of phenolic metabolites to the basal area (Fig. 3C). Results obtained by SEM and light microscopy provide confirmation of the presence of secondary metabolites in setae (Fig. 3D). The crystalline appearance of liberated material in desiccated specimens is suggestive of glycosidic compounds rather than volatile terpenoids. This corresponds with well-documented reports of phenol and terpenoid glucosides isolated from *Paederia scandens* (Quang et al., 2002) and *Erythrina indica* (Yadava and Reddy, 1999). Terpenes are stored by the plant in the form of glycosides. When required, the aglycones can be mobilized for their respective functionality in the plant (Lal et al., 2003).

L-GSTs are the most abundant trichome type and thus terpenoids isolated through distillation most probably originated from these structures. These structures are subjected to rapid loss of volatile terpenoid content after harvest, as described by Combrinck et al. (2006). Typical essential oil yields obtained from *L. scaberrima* are in the region of 0.25 % (w/w) on air-dried material. The dark colour, typifying terpenoids, obtained with vanillin–HCl is due to the mixed colour reactions of individual terpenoids because each has a distinctive colour after treatment with this reagent. Noticeable differences in shades of brown of L-GSTs observed using vanillin–HCl and UV were encountered. Similar differences in intensity using BF, although subtle, confirmed that these were variations in concentration rather than constitution. On mature leaves, limited autofluorescence of these glands can be ascribed to the presence of trace amounts of phenolic compounds present in cell walls. A complete lack of autofluorescence in these structures on young leaves is possibly due to incomplete physiological development of the head cells (Table 1). A lack of fluorescence with Natural Product reagent was indicative of an absence of phenolic compounds in the content of these structures.

In contrast to the terpenoid content of L-GSTs, S-GSTs were observed to contain phenolic compounds, which are less volatile. Possible differences in functionality within the S-GSTs group, as observed through morphological differences between un- and multicellular glands, were accentuated by the range of chemical reactions with the staining reagents used. The intensified autofluorescence of phenolic compounds obtained after treatment with Natural Product reagent offers the additional advantage that the colour reaction confirms the nature of the compounds present (Wagner and Bladt, 1996). In mature leaves the numerous S-GSTs varied in shades of orange and yellow, indicating flavone and flavonol content characteristics (Table 1).

A higher density of blue autofluorescent glands was observed on younger leaf surfaces. Observations made during this study suggest that the content of some S-GSTs are metabolically altered during leaf maturation and will influence the chemical composition of leaf extracts obtained at different seasonal intervals. According to Wagner and Bladt (1996), flavonoid extracts often contain phenol carboxylic acids (cinnamic acids) such as caffeic acid and chlorogenic acid, and coumarins, which exhibit light blue fluorescence. Some phenol carboxylic acids are precursors to more complex secondary metabolites such as flavonoids (Dewick, 1997). However, cytological allocation of phenolic compounds could not be assessed *in situ* using microscopy.

Desiccation of the material proved to be useful in distinguishing between the various S-GSTs. The valve observed in some S-GSTs (Fig. 2F) may modulate the movement of non-volatile secondary metabolites between the gland and the adjacent epidermal cells. It can be assumed that this would result in the observed rapid desiccation of these S-GSTs within 24 h post-harvest (Fig. 4E). The thick cutin barrier observed in other S-GSTs may provide resistance against withering of glandular structures and resultant retraction of glandular content into underlying tissue, leaving the gland fully distended for prolonged periods after harvest (Fig. 4D). Regardless of morphological changes, chemical changes of the content of all the S-GSTs were not apparent, as the fluorescence remained consistent when BF was used.

The presence of lipid compounds in palisade tissue is not unique in the Verbenaceae family. Such internal glandular cells are utilized by the plant as storage sites as described by Moura et al. (2005) in *Lantana camara*, a closely related plant species. However, as far as could be established, no previous reports of such secretory idioblasts in *Lippia* species have been published. Further studies to elucidate the chemical nature of the contents of such internal secretory cells should include alternative staining reagents such as Sudan Red B (lipids) and Lieberman–Buchard (triterpenes) (Moura et al., 2005) and the use of transmission electron microscopy.

**CONCLUSIONS**

Of the more than 200 known species in Verbenaceae, only about 46 have been chemically examined, with emphasis on the essential oil compositions (Pascual, 2001; Catalan and de Lampasona, 2002). In addition, investigations concerning the non-volatile components of the genus *Lippia* have been described as scarce and fragmented (Pascual, 2001). This study has therefore contributed to the knowledge of the morphology, development and contents of oil-bearing glands and will lend support to further studies of the chemical constituents of *L. scaberrima*. The combination of
SEM and light and fluorescence microscopy in a study of glandular trichomes is unconventional. It is in keeping with the current trend to use in vivo staining techniques in morphology research because it is useful to link glandular morphology and chemical functionality.

The finding that drying causes evacuation of the glandular content due to loss of volatile terpenoids is in accordance with oil yield results obtained in a previous study (Combrinck et al., 2006). This allows prediction of the optimum post-harvest drying period prior to distillation. The persistence of phenolic compounds within the plant material indicates that the medicinal use of dried leaves is a valid option and specific phenolic compounds need to be identified through isolation and structure elucidation.

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